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Notes on cultures of Exobasidium Andromedæ and of Exobasidium Vaccinii.

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WITH PLATE VI.

Among the various species of Exobasidium there has been one described on *Andromeda ligustrina* characterized mainly by the large hollow distortions which it produces on its host. Peck, who is the authority for this species, has given the original description of it in one of his reports[1] and the few other references by different botanists are all brief or even limited to the citation of the name in local floras. Unlike *Exobasidium Azaleæ* Peck, which some consider a variety of *E. Vaccinii* (Fuckel) Woronin, *E. Andromedæ* Peck has never been questioned as a distinct species and this mainly on the character of the distortion it produces. It is true that the measurements of the spores as given by Peck are much larger than those of any other Exobasidium but in all the specimens examined by the writer, including those in exsiccatæ, no such large spores were found.

In order to determine something more about the nature of this fungus and to compare it with other species of the genus an investigation was begun at the suggestion of Professor Farlow with the intention of examining all of the species of Exobasidium available. Since it will be impossible for the writer to complete this work now, and since the results of the observations and experiments already completed are definite, though unfortunately somewhat incomplete, it has seemed best to publish, at the present time, what has been done.

[1]Report State Botanist N. Y. 26: 73.

The distortions caused by Exobasidium Andromedæ, which are much the largest of any caused by the Exobasidia, hang down as large, usually greenish, bag-like distortions from the smaller branches of the host, Andromeda ligustrina. As far as could be determined the distortions arise from the young buds, either leaf or flower buds, but usually the former. They are attached by a very small point and easily break off. In size the bags vary anywhere from five to six inches long by four across, to small distortions not over two inches in their longest diameter. The color, while often more greenish than other Exobasidia, sometimes is the characteristic pink or red that one sees in E. Vaccinii on Gaylussacia.

In structure the mature gall is hollow with comparatively thin walls which are supported by numerous more or less branched cords of tissue that radiate from the point of attachment and terminate on the inner surface of the wall. With the exception of these cords and a certain amount of loose tissue hanging to them, the fully grown distortion is quite empty, contrasting with the form of E. Vaccinii on *Rhododendron viscosum* commonly known as E. Azaleæ. In the young condition the distortions of E. Andromedæ are solid and almost succulent like those of the other form referred to.

The cords which traverse the interior of the gall are nothing more than the distorted vascular bundles to which hangs a certain amount of loose parenchymatous tissue. The cells of the latter are very much elongated and very thin walled. Sometimes they even branch (fig. 12), forming a mesh work of filaments, often of considerable extent. In the younger distortions this parenchymatous tissue is more solid, and it may be said indeed to represent the distorted leaf parenchyma, but, as the gall increases in size, the cells are torn apart and, ceasing to multiply, leave the interior of the distortion hollow. As far as the vascular bundles are concerned, while they are very much split up and twisted, the spiral ducts and other wood elements individually present no very abnormal appearance.

The wall, which in the mature specimens is really nearly all there is to the distortion, consists of a number of layers of rounded parenchyma cells, tightly packed near the surface and gradually merging into the elongated cells below (fig. 1). The epidermal cells themselves, while enormously increased

in number, retain their normal size, but the others are considerably enlarged. It is to the excessively rapid growth of the cells on the periphery and almost entire quiescence of those in the interior that the hollow character of the gall is due.

The hyphæ of the parasite are mainly located in the wall of the distortion but some are seen among the parenchyma cells which cling around the vascular bundles. They are very fine, not more than 1.5μ in diameter, and are much branched, but never seem actually to enter the cells. The basidia which arise from them push their way up in the usual fashion between the cells of the epidermis. They arise as outgrowths directly from the hyphæ, with which their connection may be easily demonstrated (figs. 7, 8, 9). In each basidium a well defined nucleus is seen and its division and the subsequent formation of the spores were not seen to differ at all from that described by Rosen[2] in other Basidiomycetes. For further details one should consult this article and also the papers by Wager.[3] Of the general course of the formation of the spores Woronin has given a description, which corresponds to the figures here shown (figs. 2–6). As in other Exobasidia the number of spores on a single basidium is not constant, varying usually from four to seven, while in rare cases two were observed.

As far as the writer has seen the spores do not differ materially from those of Exobasidium Vaccinii and are subject to as much variation. They have the same elongate, sometimes slightly curved shape and finely granular contents. The size as measured in the specimens examined is scarcely more than that of the spores of E. Vaccinii, being about 14–15μ long by 3μ wide. This does not correspond, it is true, to the published descriptions, which give the size as 22–25μ long. That the spores measured were mature there can be no doubt, for many of them had become divided after the manner of the ripe spores of Exobasidia. In the specimens of Exobasidium Andromedæ in Ellis[4] the longest spore obtained was 18μ but the majority were of the size already given.

In this connection attention may be called to a misprint in Saccardo's Sylloge Fungorum[5] and in Winter's Pilze[6] where

[2]Cohn's Beitræge **6:** 259.
[3]Annals of Botany **7:** 489. 1893.—**8:** 321. 1894.
[4]N. A. F.
[5]**6:** 664.
[6]Rabh. Krypt. Flora **1[4]:** 322.

the measurement of the spores of E. Vaccinii is given as 5–8μ. On turning to Woronin's paper,[7] to which both of these descriptions are referred, it is seen that the measurement given by him is 14–17 × 3μ.

Besides the basidiospores there are found certain forms of conidial fructification. The first form is like that described by other writers on Exobasidium Vaccinii and consists of small acicular spores borne on much branched hyphæ among the basidia. The conidia of the second form are larger and are borne singly on rather stout hyphæ not occurring with the basidia (fig. 11). In germinating both of these forms send out the small acicular secondary spores, like those produced in the germination of the basidiospores. The large hollow distortions are also found on other species of Andromeda. Specimens of an Exobasidium on *Andromeda floribunda* were sent me by Dr. W. C. Sturgis, collected by him in West Virginia, which resembles exactly in all respects the E. Andromedæ.

There occurs on Andromeda ligustrina another form of Exobasidium which closely resembles in general appearance the form figured by Woronin for E. Vaccinii[8] on *Vaccinium Vitis-Idaea.* A microscopical examination went further to confirm the impression of the similarity of these two forms. Figure 17 shows in a diagrammatic outline how a section of one of these distortions resembles Woronin's figures.[9] There is not a very great derangement of the leaf tissues; the palisade cells are somewhat enlarged and the parenchyma considerably thicker than is normal. The hyphæ and basidia are in no way different from those of E. Vaccinii, nor for that matter from those of E. Andromedæ. The close resemblance of this distortion to that of E. Vaccinii on one hand and the fact that it occurred on the same host as E. Andromedæ lead the writer to suspect a possible connection between these forms and cultures on living plants were undertaken to establish some certainty about this matter.

At first it was attempted to raise the spores on nutrient media and for this purpose cultures were made on agar with various substances for the nutritive base. In agar made with a decoction of young leaves of various ericaceous plants it was possible to get the spores to germinate and produce the small

[7] Verhandl. Nat. Ges. z. Freiburg 4: —. [Heft 4.]
[8] l. c. *pl. 1. figs. 3–8.*
[9] l. c. *pl. 2. figs. 1–4.*

fusiform secondary spores, such as Woronin[10] and Brefeld[11] figure, which kept on multiplying for some time and then finally refused to grow any more. Transfers of these on live plants produced no results, so the plan was abandoned for the time being and attention was turned to making inoculations on living plants from fresh material.

For the cultures on live plants healthy young specimens of Andromeda ligustrina, Rhododendron viscosum, and Gaylussaccia resinosa entirely free from any signs of Exobasidia were selected. The experiments were carried on at Newport, R. I., during the summer of 1894, the plants having been selected the previous year so that they would be well established. During the course of the experiments the plants were kept in a glass case excluded from accidental infection and separated from each other. Beginning on the 10th of July, and for a number of days following, infections were made on these plants with the spores of various Exobasidia.

The specimens of Exobasidium used were selected with great care, only perfectly fresh ones which were as far as possible removed from contamination with other spores being collected. As soon as picked they were placed in sterilized tin boxes and so kept until the inoculation was made. As an additional safeguard specimens were not taken from one locality only, but from as wide a range of territory as possible.

The first infections were made with Exobasidium Andromedæ the surface of the distortion being scraped with a sterilized platinum spatula moistened with a drop or two of boiled water. The spores thus obtained were examined in a sterilized Van Tieghem cell and afterwards transferred to the buds and young leaves of the plants to be inoculated. In this way several plants of Andromeda, Rhododendron, and Gaylussaccia of the species already named were infected. The plants thus prepared were then isolated in a moist chamber to await developments. This treatment was repeated on other specimens.

In the same way other plants of the same kind were inoculated with spores of Exobasidium Vaccinii and also duly isolated. The experiments on Rhododendron viscosum failed for some reason or other and we will turn our attention to those on the other plants which were more successful.

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[10] l. c. *pl. 3. figs. 10–13.*

[11] Untersuch. aus d. Gesammtgeb. der Myk. 8: 9. *pl. 1. figs. 17–22.*

About ten days after the inoculation of the Andromeda plants with the spores of E. Andromedæ, there were noticed on the leaves faint discolorations, at first yellowish and later turning to pink. About five days later, the spots, which had considerably enlarged, began to show unmistakable signs of thickening, forming the familiar concavities in the leaves seen in other Exobasidia. In external form, and also in the matter of basidia and spores which will be discussed later, this distortion resembled precisely the leaf form on Andromeda ligustrina which had been collected previously. This indicates the fact that the Exobasidium Andromedæ which produces the large distortions of the young buds is identical with the leaf form found later in the summer.

The transfers on Gaylussaccia from the E. Andromedæ gave no definite results, perhaps owing to a number of mishaps to which these specimens were unfortunately subjected.

The infections made from E. Vaccinii now to be spoken of were, however, especially instructive. Those on Gaylussaccia developed in the way one would expect, the distortion on the leaf being of the kind collected out of doors near the end of the season. The most critical and interesting cultures, however, were those of this species on the Andromeda. The necessity of being especially careful with these cultures being evident, the writer adopted all possible precautions to avoid contamination. As in other cases, control specimens which had not been infected were kept under precisely similar conditions, without anything appearing on them. After about the same lapse of time as in other cases there appeared on the Andromeda leaves infected with E. Vaccinii, distortions very similar to those seen on Gaylussaccia and also to the leaf form on Andromeda which has already been described. Basidia and spores were produced from the mycelium concerning which more will be said later. That this distortion, so exactly similar to the one produced by the inoculations of the leaves of Andromeda with spores of the so-called Exobasidium Andromedæ, should be also formed by the growth of undoubted Exobasidium Vaccinii on the same host is very strong evidence of the identity of the two forms.

But before proceeding further in this discussion it will be well to speak of the microscopical appearances presented by these distortions, the description of which has been purposely left until now because of their great similarity. In their gen-

eral form all of these distortions resulting from the growth of artificial inoculations closely resemble the form of Exobasidium Vaccinii on Vaccinium Vitis-Idaea described by Woronin,[12] and the forms collected by the writer on the leaves of Andromeda and Gaylussaccia (figs. 17–20). Microscopically considered the character of the distortion on the Andromeda, from the infections of both the E. Andromedæ and the E. Vaccinii, is so precisely similar to that already described as collected in the field on the leaves of Andromeda that a description of them would be a mere repetition of the facts therein given. The mycelium, basidia, and spores likewise present no peculiarities which can separate them from those of E. Vaccinii. The spores measure 12–16 × 3μ and have the characteristic shape and appearance of those of the last named fungus (figs. 13–16).

Summing up the case, it is something like this. As has been shown above, aside from the form of the distortion, *Exobasidium Vaccinii* (Fuckel) Woronin and *E. Andromedæ* Peck cannot well be distinguished. The former can produce the same form of distortion on both Gaylussaccia and Andromeda and the latter has been made to produce a similar growth on Andromeda. Microscopically these forms do not differ. The natural conclusion is that these two species of Exobasidium are one and the same and the form producing large bag-like distortions on Andromeda should be considered a form of *Exobasidium Vaccinii* (Fuckel) Woronin.

It must be admitted that, at the time of writing, identity has not been completely established. To produce the typical form of E. Andromedæ with inoculations of spores of E. Vaccinii would be very desirable, but probably exceedingly difficult owing to the season of the year when the inoculations would have to be made. It ought to be perfectly easy however to grow distortions on Gaylussaccia from specimens of the so-called E. Andromedæ and it was the failure of these experiments which is most to be regretted.

The difference in size and structure of the distortions as a basis of specific distinction does not seem to have much weight. It is evident that the larger distortions are produced only in the early part of the year when the fungus attacks the young and sensitive tissue. In the forms ordinarily acknowledged to be Exobasidium Vaccinii there appears first the large distortion on Rhododendron viscosum formerly known as E. Azaleæ, and also on Gaylussaccia resinosa in the earliest

[12] l. c., p. 5. *pl. 11. figs. 1–6.*

formed distortions, whole shoots are transformed.[13] Later in the season the Exobasidium forms only slight local distortions on the leaves, and still later one finds forms which do not distort the tissues of the host plant at all, but simply form a scurf on the lower side of the leaves. The same succession is found in the forms on Andromeda down to the last mentioned, to which my attention was called by Dr. W. A. Setchell at Woods Hole, Mass., late in the month of August. It was also found up to early September in Newport, R. I. This form, which appears as simply slight whitish spots on the under side of the leaves, has not been definitely connected with the others as yet by means of cultures, but the spores and basidia are the same as those of the other forms on Andromeda.

This being the case it would appear that, in Exobasidium Vaccinii at least, the form and extent of the hypertrophy depends both on the host and the age of the tissues affected. The older tissues do not respond so readily to the stimulation of the parasite, and the result is a more local hypertrophy or none at all. Differences of these kinds may be commoner than is generally recognized, and where, in the simpler forms of fungi, the distortion plays an important part in specific distinctions only actual experiment with living cultures could determine whether two closely similar species are really distinct or not.

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EXPLANATION OF PLATE VI.

Fig. 1. Section of wall of distortion of Exobasidium Andromedæ on Andromeda ligustrina. × 210.

Figs. 2–9. Various stages in the development of the basidia and spores of E. Andromedæ. × 550.

Fig. 10. Mature and germinating spores of same. × 550.

Fig. 11. Conidial spores of same. × 360.

Fig. 12. Distorted parenchyma cells from the center of the gall. × 210.

Figs. 13–14. Basidia and spores from infection of Andromeda leaf with E. Andromedæ. × 550.

Figs. 15–16. Basidia and spores from infection of Andromeda leaf with E. Vaccinii.

Fig. 17. Outline of distortion produced by E. Vaccinii on Andromeda leaf. × 10.

Fig. 18. Same produced by E. Andromedæ. × 10.

Fig. 19. Same collected in the field. × 10.

Fig. 20. Same of E. Vaccinii on Gaylussaccia collected in the field. × 10.

*** All of the figures reduced about ⅓ in reproduction. Figures 1–16 were drawn with the aid of an Abbé camera.

[13] The writer collected a form similar to this on Andromeda polifolia at Shelbourne, N. H., in June, 1894.